

TECHNOLOGICAL DESIGN AND MULTI-ASSAY ANTIOXIDANT EVALUATION OF A QUERCETIN–RESVERATROL–BIOTIN FORMULATION

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Abstract

The present study presents the technological design, physicochemical characterization, and multi-assay antioxidant evaluation of a novel solid oral dosage form containing three bioactive ingredients: quercetin, resveratrol, and biotin. To overcome severe cohesiveness and poor flowability of the raw active mixture, microcrystalline cellulose, sodium starch glycolate, and magnesium stearate were incorporated into the final capsule filling powder (F_{cc}). Micromeritic optimization yielded free-flowing powder behavior, enabling precise encapsulation with suitable mass uniformity (348.25 ± 2.31 mg) and rapid *in vitro* disintegration (395 ± 4 s). Complementary FTIR, XRD, TG/DTG, and SEM analyses confirmed intermolecular hydrogen bonding, reduced crystallinity, and modified thermal decomposition profiles without chemical interference from excipients. Multi-assay antioxidant evaluation demonstrated superior, sustained radical scavenging and reducing power for F_{cc} compared with 2.8 mM ascorbic acid across ABTS (64.85% at 10 min), DPPH (67.27% at 40 min), and FRAP (2972 μ mol equivalent of Fe^{2+} ($FeSO_4$)/L at 30 min) assays. The selected excipients ensured optimal particle dispersion, preserving the redox synergy of the polyphenolic combination alongside biotin. Overall, the optimized hard gelatin capsules provide a highly uniform and bioactive oral delivery system against systemic oxidative stress.

Rezumat

Prezentul studiu prezintă designul tehnologic, caracterizarea fizico-chimică și evaluarea antioxidantă multi-test a unei noi forme farmaceutice orale solide care conține trei ingrediente bioactive: quercetină, resveratrol și biotină. Pentru a depăși coeziunea severă și curgerea slabă a amestecului de ingrediente active, celuloza microcristalină, glicolatul de amidon sodic și stearatul de magneziu au fost încorporate în pulberea finală pentru umplerea capsulelor (F_{cc}). Optimizarea micromeritică a produs o curgere liberă a pulberii, permițând o încapsulare precisă cu o uniformitate adecvată a masei ($348,25 \pm 2,31$ mg) și o dezintegrare rapidă *in vitro* (395 ± 4 s). Analizele complementare FTIR, XRD, TG/DTG și SEM au confirmat legăturile de hidrogen intermoleculare, cristalinitatea redusă și profilurile de descompunere termică modificate, fără interferențe chimice din partea excipienților. Evaluarea antioxidantă multi-test a demonstrat o putere superioară, susținută, de captare și reducere a radicalilor pentru F_{cc} comparativ cu acid ascorbic 2,8 mM în testele ABTS (64,85% la 10 min), DPPH (67,27% la 40 min) și FRAP (2972 μ mol echivalent Fe^{2+} ($FeSO_4$)/L la 30 min). Excipienții selectați au asigurat o dispersie optimă a particulelor, păstrând sinergia redox a combinației polifenolice alături de biotină. Per total, capsulele gelatinoase tari optimizate oferă un sistem de administrare orală stabil, uniform și bioactiv împotriva stresului oxidativ sistemic.

Keywords: quercetin, resveratrol, biotin, polyphenols, hard gelatin capsules, powder flowability, antioxidant activity

Introduction

Oxidative stress, defined as a profound imbalance between excessive production of reactive oxygen species (ROS) and the defensive capacity of endogenous antioxidant systems, represents a central pathophysiological pillar in the etiology and progression

of chronic non-transmissible pathologies. The superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($HO^{\bullet}$) exert cytotoxic effects through peroxidation of membrane lipids, oxidative degradation of structural and functional proteins, and induction of strand breaks in cellular DNA (1).

At the intersection of cardiology and oncology, attenuating the cardiotoxicity induced by conventional antineoplastic therapies—a pathogenic process mediated predominantly by oxidative stress and mitochondrial damage at the cardiomyocyte level—highlights the importance of incorporating molecules with antioxidant and cardioprotective potential into modern cardio-oncology strategies (2).

In this context, the use of bioactive molecules with proven antioxidant properties has evolved from a simple nutritional intervention to targeted pharmaceutical formulations capable of neutralizing free radicals and modulating redox-sensitive signalling pathways. Combining natural polyphenols with essential metabolic cofactors offers a valuable therapeutic strategy, exploiting pharmacodynamic synergy to maximize tissue protection and stabilize the cellular matrix (3).

Among the most studied polyphenolic compounds are quercetin, a flavonol from the flavonoid class, and resveratrol, a stilbenoid with remarkable pleiotropic properties. Quercetin exerts a direct antioxidant effect by scavenging reactive oxygen species and chelating transition metal ions, as well as an indirect effect by activating the Nrf2/ARE (nuclear factor erythroid 2-related factor 2) pathway, stimulating the expression of phase II antioxidant enzymes such as superoxide dismutase, catalase, and heme oxygenase-1 (4, 5).

At the cardiovascular level, therapeutic doses of quercetin prevent oxidation of low-density lipoproteins, improve endothelial function by stimulating nitric oxide synthesis, and attenuate vascular inflammation by inhibiting the NF- κ B pathway (6-8). In parallel, resveratrol enhances these responses by activating sirtuin 1 (SIRT1) and AMP-activated protein kinase (AMPK), stimulating mitochondrial biogenesis and reducing endoplasmic reticulum stress in cardiomyocytes (9,10). The combined cardioprotective effect of the two polyphenols helps limit post-ischemic ventricular remodelling, attenuate cardiac hypertrophy, and inhibit uncontrolled platelet aggregation (11).

The profound impact of oxidative stress and chronic inflammation on tumor initiation, promotion, and progression justifies the growing interest in combining quercetin and resveratrol in oncology. These compounds exert chemopreventive and pro-apoptotic effects by downregulating anti-apoptotic proteins of the Bcl-2 family, activating caspase-3 and caspase-9, and stopping the cell cycle at the G1/S or G2/M phases in multiple tumor cell lines (12-14). In addition, their anti-angiogenic properties, expressed through inhibition of vascular endothelial growth factor, block tumor neovascularization and limit the invasive and metastatic potential of malignant cells (15).

Biotin is an indispensable cofactor for biotin-dependent carboxylases, such as acetyl-CoA carboxylase and pyruvate carboxylase, which are essential for gluconeogenesis, lipogenesis, and branched-chain amino acid catabolism (16). By supporting mitochondrial function and cellular energy homeostasis, biotin complements the redox-protective action of polyphenols, helping prevent metabolic deficits induced by severe oxidative stress (17).

Formulating the combination of quercetin, resveratrol, and biotin as hard gelatin capsules is an optimal technological option for protecting bioactive substances that are sensitive to photo-oxidative degradation and humidity. This pharmaceutical form ensures precise dosing, rapid disintegration in the gastric environment, and adequate release of the active principles for intestinal absorption (18).

Materials and Methods

Materials

Quercetin ($\geq 95\%$ purity) and resveratrol ($\geq 98\%$ purity) were acquired Shanghai Zhongxin Yuxiang Chemical Co., Ltd. (Shanghai, China,) and biotin ($\geq 98\%$ purity) from Zhejiang Shengda Bio-Pharm Co., Ltd. (Taizhou, China), all supplied via Fagron Hellas (Trikala, Greece) and used as received. Microcrystalline cellulose (Avicel® PH 102) was obtained from DuPont™ Nutrition and Health (Newark, DE, USA), sodium starch glycolate (EXPLATAB®) from JRS PHARMA GmbH & Co. KG (Rosenberg, Germany), and magnesium stearate (LIGAMED® MF-2-V) from Peter Graven NV (Venlo, The Netherlands). For the antioxidant assays, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,4,6-tri(2-pyridyl)-1,3,5-triazine (TPTZ) were purchased from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany).

Methods

Physical–chemical characterization

Fourier Transform Infrared Spectroscopy (FTIR). FTIR spectra were recorded in absorbance mode using a Nicolet 6700 spectrometer (Thermo Electron Corporation, Waltham, MA, USA) across the spectral range of 4000 - 400 cm^{-1} , utilizing KBr pellets at a spectral resolution of 4 cm^{-1} .

X-ray diffraction (XRD). Powder X-ray diffraction patterns were acquired on a PANalytical Empyrean diffractometer (Malvern Panalytical) operating at room temperature with Cu K α 1 radiation ($\lambda = 1.541874 \text{ \AA}$) in Bragg–Brentano geometry, scanning over a 2θ range of 5 - 60° with a step size of 0.02°.

Thermogravimetric analysis (TG/DTG). Thermal behavior was evaluated using a Mettler Toledo TGA/SDTA851° analyzer (Mettler-Toledo GmbH, Greifensee, Switzerland) under an air atmosphere

(flow rate: 80 mL/min) at a constant heating rate of 10°C/min.

Scanning electron microscopy (SEM). Morphological analysis was performed using a Quanta 3D field-emission scanning electron microscope (Thermo Fisher Scientific GmbH, Dreieich, Germany) operating in high-vacuum mode at an accelerating voltage of 2 kV (19).

Preformulation studies. The mixture of active ingredients used in the formulation ($F_{a.i.}$) consists of quercetin, biotin, and resveratrol in a 12.5:1:12.5 mass ratio. To guide the selection and optimization of excipients, the mechanical and rheological properties of this active ingredient combination were characterized.

Particle size distribution (PSD). PSD was evaluated using a CISA RP 10 sieve shaker (CisaCedaceria Industrial, Barcelona, Spain). A 40 g sample of the mixture was loaded onto a stack of sieves with progressively decreasing mesh sizes and agitated for 20 min, after which the fraction retained on each sieve was weighed.

Powder flowability. Flow time, flow rate and angle of repose were determined for 40 g sample passing through a standardized funnel orifice (10 mm) using an automated testing system (PTG-S3, Pharma Test Apparatebau GmbH, Hainburg, Germany).

Compressibility and compactness. Bulk density (ρ_{bulk}) and tapped density (ρ_{tapped}) were determined using a tap density tester (Vankel Industries Inc.,

Cary, NC, USA) by measuring the initial volume occupied by 40 g of powder and the final volume after mechanical tapping to constant volume (1250 taps). Compressibility behavior was quantified via Carr's Index (CI) and Hausner Ratio (HR):

$$CI(\%) = 100 \times \frac{\rho_{\text{tapped}} - \rho_{\text{bulk}}}{\rho_{\text{tapped}}} \quad (1);$$

$$HR = \frac{\rho_{\text{tapped}}}{\rho_{\text{bulk}}} \quad (2).$$

According to Ph. Eur. 2.9.36. specifications, an HR < 1.25 indicates free-flowing properties, whereas a CI < 10% reflects excellent powder flowability and compactness.

Moisture content. Loss on drying was quantified using an HR 73 halogen moisture analyzer (Mettler-Toledo GmbH, Greifensee, Switzerland) at 105°C until a constant mass was reached (20).

Formulation and preparation of the filling material for capsules

Excipient selection was optimized based on preformulation results to ensure adequate flowability, compressibility, and biopharmaceutical performance, while maintaining a target fill mass suitable for size “1” hard gelatin capsules. The objective was to create capsules that patients could readily swallow and accept; therefore, maintaining a moderate, reasonable bulk was also essential. The effectiveness of the active components, which has already been demonstrated and published, was used to determine the amount per dosage unit (21). Table I presents the formulation that was evaluated ($F_{c.c.}$).

Table I

The capsule's content composition ($F_{c.c.}$)

Ingredients	Quantity (mg)/capsule	Quantity (%) /capsule
Quercetin	125	35.7
Biotin	10	2.9
Resveratrol	125	35.7
Avicel PH 102—Microcrystalline cellulose	79.5	22.7
Explotab®—Sodium starch glycolate	7	2
Ligamed® MF-2-V—Magnesium stearate	3.5	1
Total	350	100

Given the poor intrinsic flow properties of the active ingredient combinations, microcrystalline cellulose (Avicel® PH 102) was incorporated as the primary filler and flow aid. Sodium starch glycolate (EXPLATAB®) was selected as a superdisintegrant, and magnesium stearate (LIGAMED® MF-2-V) was used as the lubricant (22). All components were passed through a 20-mesh (850 μm) sieve and weighed according to Table I. The active ingredients, microcrystalline cellulose, and sodium starch glycolate were blended in a CMP 12 Plexiglas cube mixer (Pharmag GmbH, Klipphausen, Germany) at 30 rpm for 12 minutes at ambient conditions (22°C, 45% RH). Magnesium stearate was added subsequently, and blending was continued for an additional 3 minutes to prevent over-lubrication.

Evaluation of final powder blend

The final capsule powder blend was re-evaluated for particle size, moisture content, flowability, and compressibility using the methods described previously. To confirm that the antioxidant potential of the bioactive compounds was preserved in the presence of formulation excipients, the total antioxidant capacity of the complex filling powder was evaluated using three complementary assays (ABTS, DPPH, and FRAP). Ascorbic acid at a concentration of 2.8 mM (0.49 mg/mL) served as a positive control to determine whether the multi-component formulation could achieve superior radical scavenging capacity.

Sample preparation for antioxidant assays

Prior to antioxidant testing, an accurately weighed amount of $F_{c.c.}$ (equivalent to 10 mg of active polyphenols) was dispersed in 10 mL of ethanol. The mixture was sonicated for 15 minutes to ensure complete extraction of quercetin and resveratrol, and subsequently centrifuged at 4000 rpm for 10 minutes to pellet the insoluble microcrystalline cellulose. The clear supernatant was appropriately diluted and used for ABTS, DPPH, and FRAP assays. Sample blanks were used to correct for any background absorbance. **ABTS radical scavenging assay.** The ABTS radical cation ($ABTS^{\cdot+}$) was generated by reacting ABTS with potassium persulfate in the dark for 12 h at room temperature, yielding an initial absorbance of 0.70 ± 0.02 at 734 nm. After sample addition, the decrease in absorbance at 734 nm was monitored for 1 min. Results were calculated against a Trolox calibration curve and expressed as mmol Trolox equivalents per mg of formulation (23).

DPPH radical scavenging assay. Sample absorbance was measured at 517 nm every 5 min for a total duration of 40 min. Radical scavenging activity (RSA%) was calculated as:

$$RSA(\%) = \frac{Abs_0 - Abs_p}{Abs_0} \times 100 \quad (3),$$

where Abs_0 represents the initial absorbance of the DPPH working solution and Abs_p is the absorbance of the sample at time t (24).

Ferric reducing antioxidant power (FRAP) assay. Absorbance of the sample–FRAP reagent mixture was measured at 593 nm after 5 and 30 minutes of incubation. Results were interpolated from an Fe^{2+} ($FeSO_4$) calibration curve and expressed as $\mu mol Fe^{2+}$ equivalents/L (25).

Hard gelatin capsules manufacturing and quality control. The final powder blend was filled into opaque white, size 1 hard gelatin capsules (target fill mass: 350 mg) using a semi-automatic encapsulator (FagronLab™ FG1, Gako Konietzko GmbH, Bamberg, Germany). Given the tapped density of 0.713 g/mL, 350 mg of powder occupies approximately 0.49 mL, fitting the volume of a size 1 capsule

(~0.50 mL) using minimal tamping pressure. Quality control testing of the capsules was performed in accordance with European Pharmacopoeia standards (26).

Uniformity of mass. Mass variation was evaluated by individually weighing 10 filled capsules, determining the net mass of the contents after emptying the shells, and calculating the deviation relative to the mean value.

In vitro disintegration time. Disintegration was evaluated using an Erweka DT 3 apparatus (Erweka® GmbH, Langen, Germany) in distilled water at $37 \pm 0.5^\circ C$ ($n = 6$). The time required for complete disintegration, defined as the absence of undissolved mass on the discs except for capsule shell fragments, was recorded.

Results and Discussion

Physical–chemical characterization

Figure 1 shows the FTIR spectra of formulations containing active ingredients (quercetin, biotin, and resveratrol) with and without excipients. The FTIR spectrum of the formulation without excipients, $F_{a.i.}$ (Figure 1a), shows the characteristic bands associated with quercetin, biotin, and resveratrol. Several changes in the intensity and position of some characteristic bands are associated with molecular interactions between the active ingredients, mainly related to hydrogen bonding. The O–H stretching vibration of quercetin (3446 cm^{-1}) and of the phenolic group (1379 cm^{-1}) (27), the broad band between $3200\text{--}3500\text{ cm}^{-1}$ of resveratrol (28), and the band of biotin (3386 cm^{-1}) (19) show a slight shift in peak position in both formulations (with and without excipients), as shown in Figure 1. This suggests possible interactions between the active ingredients. Because the characteristic peaks of the main ingredients remain visible in the formulation with excipients, and only a reduction or slight displacement is observed, this suggests that the excipients do not influence the final pharmaceutical formulation.

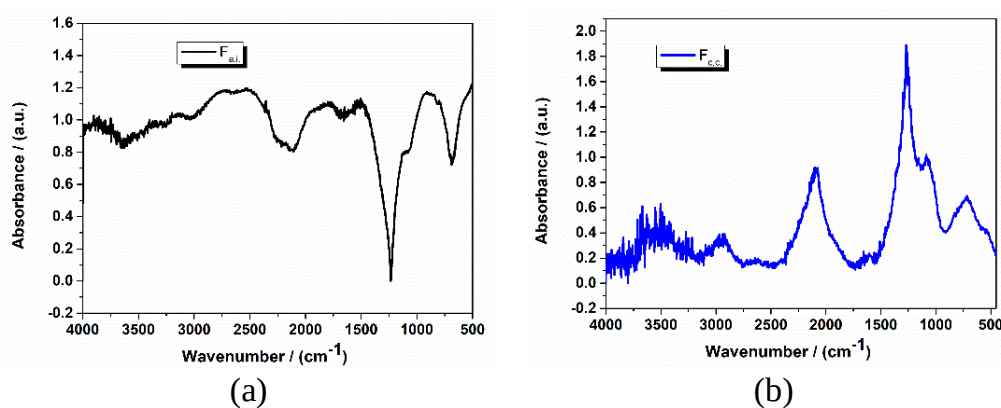


Figure 1.
FTIR analysis of (a) $F_{a.i.}$; (b) $F_{c.c.}$

In Figure 2, the X-ray diffractograms are shown for $F_{a.i.}$ (Figure 2a) and $F_{c.c.}$ (Figure 2b).

According to the literature, the main characteristic diffraction peaks of quercetin ($2\theta = 24.4^\circ$ and 27.3°), biotin ($2\theta = 17.2^\circ$ and 18.4°), and resveratrol ($2\theta = 22.5^\circ$, 23.7° , 25.4° , and 28.45°) indicate a well-organized structure, demonstrating a crystalline arrangement (29-31). The XRD spectra of the formulations in Figure 2 show the disappearance of some peaks of the main ingredients, suggesting new interactions between them. These interactions also indicate a decrease in crystallinity or a reorganization of the structure of the active ingredients within the formulations, attributed to intermolecular interactions. In

the formulation containing excipients (Figure 2b), no further modifications of the diffraction pattern were observed. The disappearance or reduction of characteristic peaks in the formulated powder $F_{c.c.}$ is predominantly due to the dilution effect of the active ingredients within the amorphous matrix of microcrystalline cellulose (Avicel PH 102), which accounts for a significant portion of the blend, rather than solely chemical interactions. Furthermore, the baseline scattering from the amorphous MCC masks the lower-intensity crystalline peaks.

The thermal curves of formulations without and with excipients are shown in Figure 3 (Figure 3a for $F_{a.i.}$, and Figure 3b for $F_{c.c.}$).

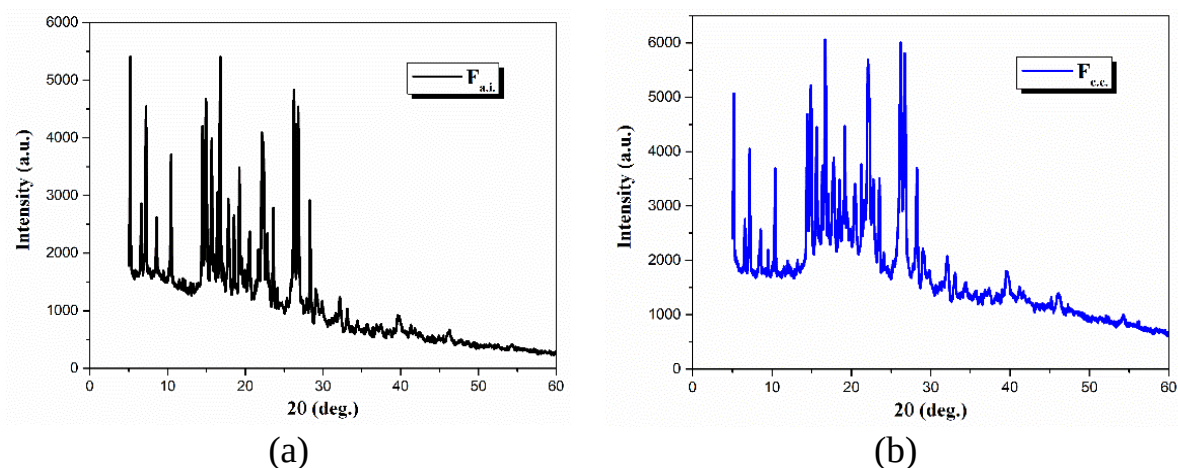


Figure 2.

XRD spectra of formulations without and with excipients (a) $F_{a.i.}$ (b) $F_{c.c.}$ in the range of $2\theta = 5 - 60^\circ$

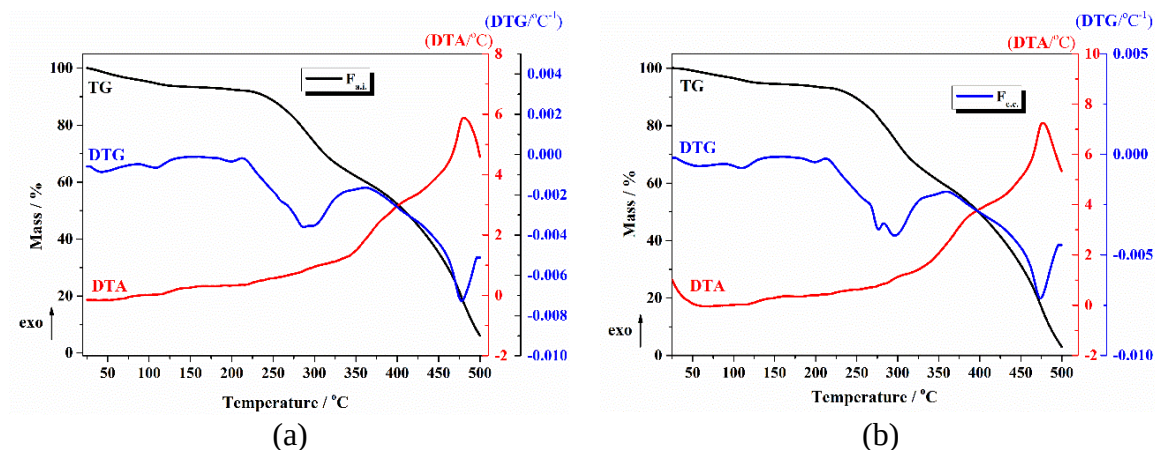


Figure 3.

TG/DTG curves of (a) $F_{a.i.}$; (b) $F_{c.c.}$ (air atmosphere, flow rate of 80 mL min^{-1} , heating rate - 10°C/min)

The thermal decomposition profiles of $F_{a.i.}$, which incorporates only the active ingredients (quercetin, biotin, and resveratrol), and $F_{c.c.}$ show the following steps: (i) an initial mass loss up to 150°C for $F_{a.i.}$ and up to 130°C for $F_{c.c.}$, due to the loss of water and solvents. The mass loss in the first step was about 6.6% for $F_{a.i.}$ and 5.1% for $F_{c.c.}$; (ii) the second decomposition step is a complex process between 190 and

480°C for $F_{a.i.}$ and 150 and 490°C for $F_{c.c.}$. This process can be attributed to the combustion of organic compounds from the formulations. It was observed from Figure 3 that the thermal decomposition of the formulation with excipients ($F_{c.c.}$) starts at a lower temperature compared with the formulation that contains only the active compounds. For both formulations, the decomposition process is not completed by

500°C. The residue at 500°C for $F_{a.i}$ is 6.67% and for $F_{c.c.}$ is 3.37%, respectively. Overall, the thermal profiles of the two formulations show distinct decomposition behavior. The presence of excipients shifts the onset of thermal decomposition to a lower temperature, which indicates a modification of the thermal stability (32). Also, the residue at 500°C is lower for the formulation with excipients compared with the formulation that contains only active compounds, demonstrating a greater influence of excipients on the thermal decomposition. Nevertheless, the thermal degradation behavior is a complex process in the case of both formulations. The lower onset of de-

composition and the lower residue in $F_{c.c.}$ are characteristic of the dilution effect caused by the addition of organic excipients (MCC, sodium starch glycolate).

In our previous research, SEM micrographs of quercetin and resveratrol showed aggregates of spherical particles, while biotin exhibited a needle-like structure (19, 33). The SEM images of $F_{a.i.}$ (Figure 4a) and $F_{c.c.}$ (Figure 4b) show fine, irregular crystals. Compared with the SEM images reported for active ingredients in our previous studies, some morphological changes are observed, mostly due to modifications in the solid-state structure, as also evidenced by XRD analysis.

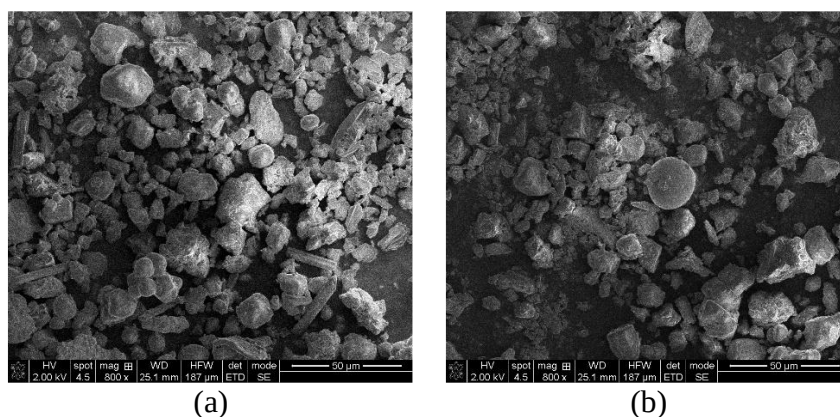


Figure 4.
SEM micrographs: (a) $F_{a.i.}$; (b) $F_{c.c.}$, at a magnification of 800×

All these complementary physicochemical findings suggest that the formulation with active ingredients based on quercetin, biotin, and resveratrol, and the subsequent incorporation of excipients, substantially modifies the molecular interactions and thermal be-

havior. The corroboration of all these results supports the successful integration of quercetin, biotin, and resveratrol into the final capsule formulation.

Preformulation studies

Figure 5 shows the histogram of the particle size distribution for the active ingredients mixture ($F_{a.i.}$) and the final capsule filling powder ($F_{c.c.}$).

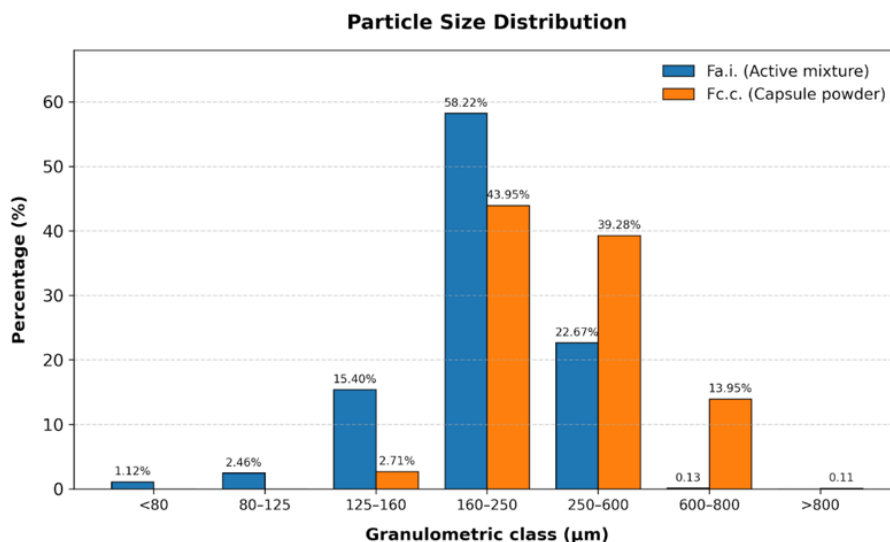


Figure 5.
The particle size distribution of the materials

A clear shift in particle size is observed after the incorporation of excipients. The active ingredients mixture ($F_{a.i.}$) exhibits a predominantly fine-to-medium granulometric profile, with most particles in the 160 - 250 μm range (58.22%), and notable fractions in the 125 - 160 μm (15.40%) and 250 - 600 μm (22.67%) ranges. Fine particles below 125 μm account for 3.58% of $F_{a.i.}$, primarily reflecting the micronized form of quercetin and biotin, while coarse particles above 600 μm are virtually absent (0.13%).

The addition of excipients in $F_{c.c.}$ induces a pronounced enlargement of the particle size distribution. The finest fractions below 125 μm are completely eliminated (0%), while the 125 - 160 μm class decreases markedly from 15.40% to 2.71%. Concurrently, the proportion of larger particles increases substantially: the 250 - 600 μm class rises to 39.28%, and a substantial coarse population appears in the 600 - 800 μm interval (13.95% compared to 0.13% in $F_{a.i.}$).

This upward shift in granulometry is attributed to the inclusion of microcrystalline cellulose (Avicel® PH 102), which has a larger intrinsic average particle size and a fibrous morphology that promotes particle interlocking and masks the cohesive fine active powders. This broader, coarser particle size distribution in $F_{c.c.}$ is highly advantageous for formulation processing, as it eliminates fine dust, reduces interparticle cohesion, and enhances the volumetric flowability required for uniform capsule filling (34).

The shift towards larger particle sizes ($> 250 \mu\text{m}$) in the final blend, despite Avicel PH 102 typically having a nominal particle size of $\sim 100 \mu\text{m}$, is the result of mechanical micro-agglomeration. During the 15-minute blending process, the highly cohesive fine particles of quercetin and resveratrol adhered to the surface of the MCC particles and to each other, acting as weak solid bridges.

The rheological parameters of the active ingredients mixture ($F_{a.i.}$) and the final capsule filling powder ($F_{c.c.}$) are summarized in Table II.

Table II

Flowing parameters of the samples

<i>Parameter</i>	<i>F_{a.i.}</i>	<i>F_{c.c.}</i>
Flow time (sec)	28.7 ± 1.55	17.7 ± 2.01
Angle of repose ($^\circ$)	42.66 ± 2.04	27.35 ± 1.82
Flow rate (g/s)	1.393	2.259

The active ingredients mixture ($F_{a.i.}$) exhibited poor flow properties, characterized by a prolonged flow time (28.7 ± 1.55 s), a low flow rate (1.393 g/s), and a high angle of repose ($42.66 \pm 2.04^\circ$). In accordance with pharmacopoeial standards, an angle of repose exceeding 40° indicates cohesive behavior and poor flowability (26), driven by strong interparticle attractive forces and the electrostatic nature of the unfor-

mulated bioactive compounds (quercetin, resveratrol, and biotin). Mechanical agitation (stirring at 25 rpm) was required to achieve continuous flow of $F_{a.i.}$ through the testing funnel, confirming its intrinsic resistance to free movement.

Conversely, the addition of the selected excipients significantly improved the dynamic properties of the powder blend ($F_{c.c.}$). The flow time sharply decreased to 17.7 ± 2.01 s, accompanied by a 62% increase in flow rate (2.259 g/s) without mechanical stirring. Most notably, the angle of repose dropped to $27.35 \pm 1.82^\circ$, shifting the powder behavior directly from poor/cohesive ($> 40^\circ$) to the excellent, free-flowing category ($< 30^\circ$).

This marked improvement in rheological behavior is attributed to the synergistic action of the excipients: microcrystalline cellulose (Avicel® PH 102) acts as the primary filler, smoothing surface irregularities and increasing bulk density, while magnesium stearate (LIGAMED® MF-2-V) reduces interparticle friction as a lubricant (35). Consequently, the final complex powder ($F_{c.c.}$) exhibits the required flowability for automatic encapsulation, ensuring uniform volumetric die filling and low mass variation in the final dosage units.

The volumetric and compressibility parameters of the active ingredients mixture ($F_{a.i.}$) and the final capsule filling powder ($F_{c.c.}$) are presented in Table III.

Table III

Volumetric properties of the samples

<i>Parameter</i>	<i>F_{a.i.}</i>	<i>F_{c.c.}</i>
Bulk density (g/ml)	0.509 ± 0.18	0.567 ± 0.11
Tapped density (g/ml)	0.830 ± 0.26	0.713 ± 0.24
Carr Index (CI) (%)	38.674	20.47
Hausner's ratio (HR)	1.630	1.257

According to European Pharmacopoeia classification criteria, the active mixture ($F_{a.i.}$) exhibited characteristics of very poor flowability and extreme cohesiveness, with a Carr Index (CI) of 38.674% and a Hausner Ratio (HR) of 1.630. The low initial bulk density (0.509 ± 0.18 g/mL) relative to its high tapped density (0.830 ± 0.26 g/mL) indicates a large volume of interparticle void spaces that collapse significantly under mechanical vibration, reflecting strong interparticle friction and cohesion among unformulated quercetin, resveratrol, and biotin particles.

Incorporation of the selected excipient system resulted in a notable redistribution of the volumetric profile in $F_{c.c.}$. While bulk density increased moderately to 0.567 ± 0.11 g/mL, reflecting better initial packing efficiency, tapped density decreased markedly to 0.713 ± 0.24 g/mL. Consequently, the CI value dropped significantly from 38.674% to 20.470%, and the HR decreased from 1.630 to 1.257.

This favorable shift transitions the mechanical behavior of the powder blend from a “very poor” rating directly into the “fair/passable” category suitable for pharmaceutical manufacturing. The effective combination of microcrystalline cellulose (Avicel® PH 102) and magnesium stearate (LIGAMED® MF-2-V) reduces interparticle adhesion and friction, limiting excessive consolidation during tapping. The resulting compressibility attributes of $F_{c.c.}$ ensure adequate mechanical stability and volumetric consistency during the semi-automatic capsule-filling process.

The moisture content analysis revealed a loss on drying of 4.72% for the active ingredients mixture ($F_{a.i.}$), reflecting the intrinsic hygroscopicity and residual bound moisture associated with the raw bioactive compounds. Following incorporation of the excipients, a notable decrease in moisture content was observed in the final powder blend ($F_{c.c.}$), dropping to 2.93%.

This substantial reduction is primarily attributed to the dilution effect introduced by low-moisture, pharmaceutical-grade excipients (microcrystalline cellulose and magnesium stearate). Maintaining a moisture level below 3% in $F_{c.c.}$ is highly advantageous for hard capsule formulations, as it minimizes the risk of hydrolytic degradation of sensitive bioactive components, prevents particle agglomeration due to liquid capillary bridging (supporting enhanced flowability), and ensures long-term physical and chemical stability during storage (36).

The physical and micromeritic performance of solid pharmaceutical dosage forms is governed by an intricate, multivariable interplay among particle size distribution, surface moisture, rheological behavior and volumetric packing mechanics. In the present investigation, a comprehensive evaluation of the transition from the active ingredient mixture ($F_{a.i.}$) to the final capsule-filling formulation ($F_{c.c.}$) demonstrates a fundamental restructuring of powder physics. The raw bioactive complex ($F_{a.i.}$), consisting of quercetin, biotin and resveratrol in a 12.5:1:12.5 mass ratio, exhibits pronounced technological limitations characterized by poor flowability, high cohesion, excessive moisture retention and unfavorable compressibility. The incorporation of functional excipients - microcrystalline cellulose, sodium starch glycolate, and magnesium stearate - redefines these mechanical attributes, establishing direct causal correlations among particle dimensions, residual water content, dynamic flow kinetics and consolidation dynamics. The primary advancement in the observed micromeritic evolution is the radical modification of the particle size distribution profile. $F_{a.i.}$ contains a significant fraction of fine particles, with 18.98% of its mass below 160 μm (including 1.12% below 80 μm and 2.46% in the 80 - 125 μm range). Fine particles possess an exceptionally high specific surface

area, which dramatically amplifies interparticle van der Waals attraction and electrostatic charging (37). Consequently, $F_{a.i.}$ exhibits severe particle-particle adhesion and cohesive arching, directly explaining its poor rheological behavior, evidenced by an angle of repose (θ) of $42.66 \pm 2.04^\circ$, a prolonged flow time of 28.7 ± 1.55 s, and a low mass flow rate of 1.393 g/s under mechanical agitation.

Following excipient addition, the particle size spectrum of $F_{c.c.}$ shifts toward larger, more uniform granulometric classes. Fine particles below 125 μm are completely eliminated (0%), while coarse fractions increase substantially, with the 250 - 600 μm class rising from 22.67% to 39.28% and a new population appearing in the 600 - 800 μm range (13.95%). This coarsening effect, primarily imparted by the microcrystalline cellulose matrix, reduces the total surface area available for interparticle friction (38-40). As a direct mathematical consequence of this particle size enlargement, the angle of repose decreases by 15.31° to an excellent value of $27.35 \pm 1.82^\circ$, accompanied by a 62% increase in gravity-driven flow rate (2.259 g/s) without the need for external mechanical vibration.

This particle size rearrangement is closely coupled to the thermodynamic state of water within the powder bed. Loss-on-drying analysis revealed a high initial moisture content of 4.72% in $F_{a.i.}$, attributed to the inherent hygroscopicity and surface-bound water of the polyphenolic compounds. At elevated moisture levels, thin adsorbed liquid films form on the surfaces of fine particles, giving rise to capillary liquid bridges and surface tension-mediated cohesive forces. These liquid bridges act as mechanical anchors that resist shear stress, restrict interparticle sliding, and contribute to the high angle of repose and slow flow kinetics observed in $F_{a.i.}$ (41, 42). In contrast, the incorporation of low-moisture excipients in $F_{c.c.}$ reduces the overall loss on drying to 2.93%. This reduction below the critical 3% threshold effectively dismantles liquid capillary networks and suppresses interparticle liquid adhesion. The combined reduction of fines and moisture in $F_{c.c.}$ minimizes both solid-state van der Waals forces and liquid-phase surface tension, enabling individual particles to slide freely past one another under gravitational force (43).

The structural impact of particle size and moisture content extends directly into the volumetric properties and compaction mechanics of the powders. In its raw state, $F_{a.i.}$ exhibits a low bulk density ($\rho_{\text{bulk}} = 0.509 \pm 0.18$ g/mL) due to cohesive particle bridging, which traps substantial volumes of air within irregular, loosely packed interparticle voids (44). However, when subjected to mechanical tapping, these fragile cohesive bridges collapse completely, forcing the fine particles into a highly consolidated structure with a high tapped density ($\rho_{\text{tapped}} = 0.830$

± 0.26 g/mL). The large consolidation gap ($\Delta\rho = \rho_{\text{tapped}} - \rho_{\text{bulk}} = 0.321$ g/mL) reflects an extreme degree of volume reduction under mechanical stress. Quantified through empirical compressibility indices, $F_{a.i.}$ yields a Carr Index (CI) of 38.674% and a Hausner Ratio (HR) of 1.630, placing the unformulated active mixture squarely in the “very poor” compressibility class according to European Pharmacopoeia standards. Such high values indicate that $F_{a.i.}$ would undergo erratic consolidation during automated capsule filling, leading to severe mass variation and inconsistent dosing (45).

The formulation strategy implemented in $F_{c.c.}$ fundamentally alters this consolidation behavior. The addition of Avicel® PH 102 introduces a microporous, fibrous polymeric skeleton that increases the initial bulk density to 0.567 ± 0.11 g/mL, establishing a denser and more stable baseline packing state (46). Simultaneously, the inclusion of magnesium stearate coats the surface of the larger particles, forming a hydrophobic lubricant film that lowers the interparticle friction coefficient (47). Consequently, when $F_{c.c.}$ is tapped, the prearranged particle matrix resists excessive consolidation, limiting the tapped density to 0.713 ± 0.24 g/mL. This reduces the consolidation amplitude ($\Delta\rho$) by more than half, down to 0.146 g/mL.

Mathematically, this diminished density differential drives a dramatic reduction in both compressibility indices for $F_{c.c.}$, yielding a Carr Index of 20.470% and a Hausner Ratio of 1.257. This shift represents a direct transition from a highly cohesive, unpredictable powder state (CI > 38%) to a passable, free-flowing system (CI $\approx$ 20%, HR $\approx$ 1.25) suitable for indus-

trial processing. The strong linear correlation between the elimination of fine particles (<125 μm), the reduction in moisture content (from 4.72% to 2.93%), the drop in angle of repose (from 42.66° to 27.35°), and the decline in Carr Index (from 38.674% to 20.470%) confirms that the physical performance of $F_{c.c.}$ is an integrated system response. The removal of fine-fraction-induced cohesion prevents air entrapment, while moisture control suppresses capillary adhesion, together enabling stable volumetric packing, rapid die filling, and uniform mass distribution during hard gelatin capsule manufacturing (48,49).

Antioxidant activity

ABTS radical scavenging assay

Evaluation of the total antioxidant capacity using the ABTS radical cation (ABTS^{•+}) decolorization assay demonstrated good radical scavenging activity for $F_{c.c.}$, significantly outperforming the standard reference antioxidant, ascorbic acid (2.8 mM), at both evaluated time intervals.

Figure 6 illustrates the comparative ABTS radical scavenging values at 5 and 10 minutes of incubation. At the initial 5-minute mark, $F_{c.c.}$ achieved a scavenging capacity of 46.33%, representing a 2.95-fold increase compared with the reference ascorbic acid solution (15.72%). As the reaction progressed to 10 minutes, both evaluated systems exhibited time-dependent quenching kinetics, reflecting ongoing electron and hydrogen atom transfer processes. However, $F_{c.c.}$ maintained its marked superiority, reaching a value of 64.85 % compared with 26.19 % for ascorbic acid, corresponding to a 2.48-fold higher efficacy in neutralizing the cationic radical species.

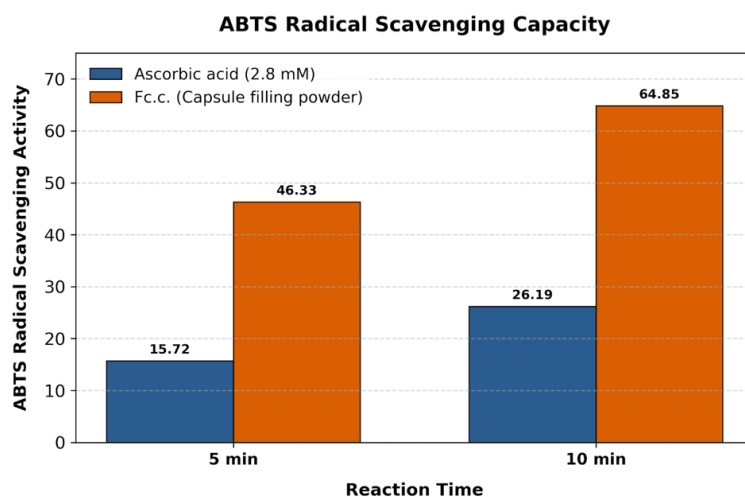


Figure 6.

The ABTS radical scavenging activity

The pronounced antioxidant potential displayed by $F_{c.c.}$ is directly governed by the synergistic chemical structures of its active polyphenolic constituents.

Quercetin exhibits exceptional single-electron transfer and hydrogen-donating properties due to its catechol structure in the B-ring and the 3-hydroxyl/4-

keto arrangement in the C-ring (50), while resveratrol contributes additional reactive phenolic hydroxyl groups capable of stabilizing radical intermediates (51). Furthermore, these findings confirm that the inclusion of formulation excipients does not induce chemical deactivation or steric hindrance of the active antioxidant centers, thereby preserving the high bioactivity of the complex powder matrix in the final solid dosage form.

DPPH radical scavenging assay

The kinetic monitoring of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity over a 40-minute reaction period revealed a pronounced, time-dependent antioxidant capability for F_{c.c.}, which markedly surpassed the scavenging kinetics of reference ascorbic acid (2.8 mM).

Figure 7 illustrates progressive radical neutralization curves measured at 5-minute intervals. At the initial 5-minute time point, F_{c.c.} demonstrated rapid-onset antioxidant activity with an RSA of 30.22%, compared to 11.06% for ascorbic acid. As the reaction progressed, F_{c.c.} showed a continuous, sustained increase in radical quenching capacity, rising to 40.14% at 10 minutes, 52.04% at 20 minutes, and reaching a maximum of 67.27% after 40 minutes of incubation. In contrast, ascorbic acid exhibited a slower kinetic profile, achieving an RSA of only 24.27% at the end of the 40-minute testing window. Throughout the entire time course, F_{c.c.} consistently maintained a 2.5- to 2.8-fold higher radical scavenging capacity than the reference antioxidant control.

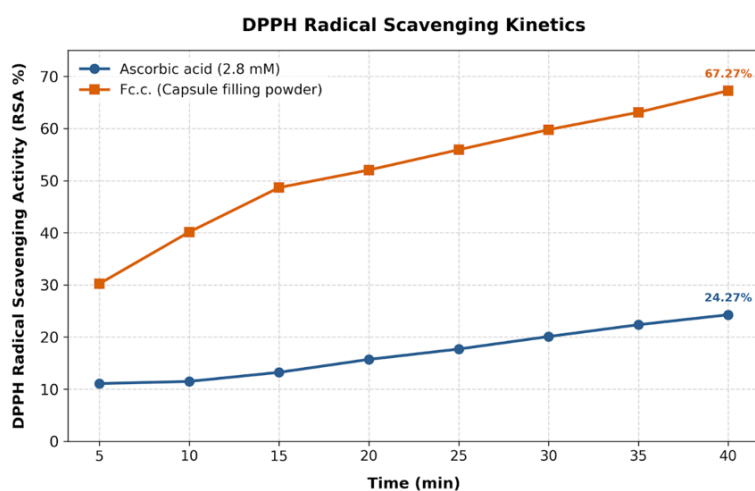


Figure 7.

The DPPH radical scavenging activity

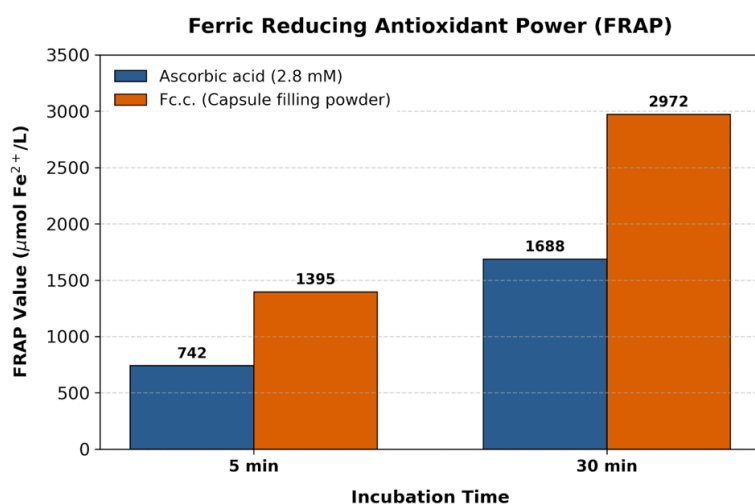


Figure 8.

The FRAP assay results

The sustained kinetic trajectory of F_{c.c.} reflects the combined hydrogen-atom transfer and single-electron transfer mechanisms provided by the multicomponent polyphenolic system. The catechol hydroxyl

groups of quercetin and the stilbenoid phenolic hydroxyls of resveratrol act synergistically to stabilize the nitrogen-centered DPPH free radical into its diamagnetic hydrazine form (DPPH-H) (52). The

steady, non-plateauing rise up to 40 minutes confirms the sustained release of active antioxidant species from the solid matrix into solution and shows that the inclusion of formulation excipients does not impede radical scavenging kinetics or sterically hinder the reactive phenolic sites (53).

FRAP assay

The total electron-donating capacity of the formulations was evaluated using the FRAP assay, which measures the ability of antioxidant compounds to reduce the colorless ferric-tripyridyltriazine complex to an intensely blue ferrous complex at low pH. Figure 8 shows the measured FRAP values for the final capsule-filling powder and the positive control at two distinct time points. In line with the trends observed in the ABTS and DPPH radical scavenging assays, the complex solid formulation exhibited a markedly higher reducing potential than the standard ascorbic acid solution at a concentration of 2.8 mM. A detailed quantitative analysis reveals the rapid and potent electron transfer kinetics of the formulated polyphenols. After 5 minutes of incubation, the final capsule-filling powder achieved a FRAP value of 1395 μmol equivalent of Fe^{2+} (FeSO_4) /L, approximately 1.88 times greater than the 742 μmol equivalent of Fe^{2+} (FeSO_4) /L recorded for the ascorbic acid standard. This difference became even more pronounced as the reaction progressed to the 30-minute mark. At this final checkpoint, the formulation reached a good reducing capacity of 2972 μmol equivalent of Fe^{2+} (FeSO_4) /L, while the ascorbic acid reference attained a value of only 1688 μmol equivalent of Fe^{2+} (FeSO_4) /L. This sustained increase over time confirms that the reduction process mediated by the active matrix is not only fast-acting but also possesses a substantial reserve of electron-donating power that continues to emerge as the formulation interacts with the oxidative environment. The important ferric-reducing ability of the complex powder is structurally rooted in the synergistic combination of quercetin and resveratrol. Both polyphenolic molecules contain multiple highly reactive hydroxyl groups that serve as excellent electron donors, effectively reducing transition metal ions. This mechanism is of major physiological importance, particularly in cardio-oncology, because the chelation and reduction of transition metals play a critical role in preventing metal-catalyzed oxidative stress, such as the Fenton reaction (54, 55). Moreover, these robust spectrophotometric results clearly demonstrate that the selected pharmaceutical excipients used to optimize powder flowability and compressibility do not trap the bioactive molecules or interfere with their electron transfer mechanisms, ensuring that the full antioxidant potential is delivered by the final capsule dosage form.

The correlation among the ABTS, DPPH, and FRAP determinations provides robust, multi-method confirmation of the superior antioxidant profile of the final capsule formulation. A consistent positive trend is evident across all three spectrophotometric assays, showing that the complex formulation operates simultaneously through hydrogen atom transfer and single-electron transfer mechanisms. In the ABTS cation radical assay, $F_{c.c.}$ achieved a rapid scavenging capacity of 64.85 at 10 minutes, closely paralleling the sustained kinetic trajectory observed in the DPPH assay, where radical scavenging activity reached 67.27% at 40 minutes. This continuous kinetic release of radical-quenching capacity is further corroborated by the FRAP assay, which recorded a ferric-reducing capability increasing from 1395 to 2972 μmol equivalent of Fe^{2+} (FeSO_4) /L. This strong inter-assay agreement indicates that the antioxidant potential is an integrated redox phenomenon capable of neutralizing both nitrogen- and oxygen-centered radicals while reducing catalytic transition metals (56).

This multi-mechanistic bioactivity directly correlates with the qualitative and quantitative composition of the active triad, consisting of quercetin, resveratrol and biotin. The high radical-scavenging metrics in ABTS and DPPH are primarily driven by the phenolic hydroxyl architecture of quercetin and resveratrol. The incorporation of biotin completes this bioactive complex. Although biotin functions fundamentally as an essential metabolic cofactor for mitochondrial carboxylases, its co-formulation with quercetin and resveratrol establishes an important synergy. By supporting cellular energy metabolism and mitochondrial integrity, biotin complements the direct chemical scavenging actions of the polyphenols, providing systemic protection against metabolic oxidative stress. The assay results confirm that biotin acts harmoniously within the active mass ratio without causing chemical inactivation or competitive inhibition of the polyphenolic reactive sites during radical quenching (57, 58).

Furthermore, correlating the chemical antioxidant performance with the overall formulation composition highlights the functional success of the excipient matrix. Microcrystalline cellulose, sodium starch glycolate, and magnesium stearate were incorporated to optimize powder flowability and compressibility without adsorbing or deactivating the active molecules. The rapid initial kinetics recorded in the first 5 minutes across all three assays demonstrate that the excipient matrix facilitates rapid particle wetting and complete spatial dispersion of the active components upon contact with the medium. Sodium starch glycolate promotes rapid hydration, preventing hydrophobic agglomeration of quercetin and resveratrol, while microcrystalline cellulose ensures

uniform particle distribution. Consequently, the steric accessibility of the active sites on quercetin, resveratrol, and biotin remains unhindered, confirming that the optimized technological properties of $F_{c.c.}$ directly preserve and maximize biochemical antioxidant performance in the final solid dosage form.

Final capsules qualitative evaluation

The quality control evaluation of the manufactured hard gelatin capsules ($F_{c.c.}$) demonstrated good volumetric filling precision and mass consistency, yielding a mean fill mass of 348.25 ± 2.31 mg ($n = 10$). All tested dosage units complied with compendial standards, with deviations remaining below the allowable $\pm 5\%$ limit.

This high degree of mass uniformity directly validates the rheological and volumetric optimizations achieved in $F_{c.c.}$. The suppression of fine particles (<125 μ m), reduction of moisture content to 2.93%, and the resulting transition to free-flowing powder dynamics ($\theta = 27.35^\circ$, $CI = 20.47\%$, $HR = 1.257$) successfully eliminated arching, air bridging, and friction within the hopper during semi-automatic encapsulation, ensuring reproducible volumetric die filling and uniform dosing across individual units (59).

The *in vitro* disintegration time of hard gelatin capsules filled with $F_{c.c.}$ was 395 ± 4 s. This performance complies with the compendial requirements for hard gelatin capsules, which stipulate that complete disintegration must occur within a maximum of 30 minutes in distilled water at 37 ± 0.5 °C. The inclusion of sodium starch glycolate plays an important role. Upon wetting, it undergoes extensive swelling without forming a viscous gel barrier, generating high internal swelling pressure that rapidly disrupts the powder core (60). Microcrystalline cellulose acts synergistically through a capillary wicking mechanism, drawing water into the porous interior of the bed and accelerating core breakup (35).

This disintegration behavior represents an essential prerequisite for the subsequent fluid contact and potential dissolution of the poorly soluble polyphenolic constituents (quercetin and resveratrol) in the gastrointestinal tract.

The technological viability of the final capsule dosage form depends on the functional synergy among the excipients, which simultaneously governs both mass uniformity and *in vitro* disintegration kinetics. Suitable mass consistency is the direct macroscopic consequence of the optimized rheological and volumetric profile produced by the excipients. Microcrystalline cellulose restructured the overall particle size distribution and increased the initial bulk packing density, while the boundary lubrication provided by magnesium stearate markedly reduced interparticle friction and eliminated the cohesive arching previously observed in the active-ingredients mixture. This precise mechanical conditioning produced a

free-flowing powder bed capable of consistent, uninterrupted volumetric feeding into the capsule dies during semi-automatic encapsulation, thereby preventing mass fluctuations and ensuring compliance with pharmacopoeial uniformity standards (61).

Conversely, while excellent flowability and compressibility ensure uniform filling, the densified powder matrix must also disintegrate rapidly upon administration to release the active compounds. The fast disintegration time confirms that the structural consolidation required for accurate dosing does not compromise the biopharmaceutical performance of the capsules. This rapid breakdown is governed by the synergistic hydrodynamics of sodium starch glycolate and microcrystalline cellulose, which effectively counteract the water-repellent boundary films formed by the magnesium stearate lubricant. Upon contact with the aqueous medium, microcrystalline cellulose promotes rapid fluid ingress into the compacted core via capillary wicking. This establishes immediate fluid pathways that trigger extensive, multidimensional hydration of the superdisintegrant. The sudden volume expansion generates high internal swelling pressure that overcomes the interparticle adhesive forces, exerting destructive mechanical stress that rapidly shatters the densified powder matrix. Consequently, the strictly controlled excipient mass ratio balances the potentially antagonistic requirements of the solid dosage form, providing sufficient rheological stability for highly uniform dosing alongside a robust disintegration mechanism essential for the prompt dissolution of the active polyphenolic constituents (62–64).

While this study successfully demonstrates the preformulation and micromeritic optimization of a multi-component dietary supplement, several limitations must be acknowledged. The current investigation was conducted on a single, lab-scale batch using semi-automatic encapsulation. Quality control was limited to compendial mass uniformity and disintegration tests, without validated HPLC assays for content uniformity (as per Ph. Eur. 2.9.40). Furthermore, the biopharmaceutical profile was assessed via chemical antioxidant assays; formal *in vitro* dissolution testing in biorelevant media and long-term ICH stability studies (evaluating photo-oxidative degradation) were not performed. Future research will address these limitations by validating analytical methods, performing multi-batch scale-up, and evaluating the *in vitro* release kinetics and long-term stability of the capsules.

Conclusions

In the present study, a novel multi-component solid dosage form containing quercetin, resveratrol, and biotin was successfully formulated, characterized, and optimized for potential applications as a dietary

supplement. The initial active ingredient complex exhibited severe technological limitations, including extreme cohesiveness, poor flowability, and high moisture retention that restricted encapsulation. By integrating suitable excipients, the particle size distribution was favorably shifted away from fine cohesive fractions, moisture content was reduced below 3%, and the powder mechanics transitioned into a free-flowing, passably compressible state suitable for semi-automatic encapsulation. Spectrophotometric evaluations using ABTS, DPPH, and FRAP assays demonstrated that the multi-component polyphenolic triad preserved its potent antioxidant capacity within the final powder matrix, significantly outperforming high-concentration ascorbic acid controls across all temporal checkpoints, while confirming that the excipients do not induce chemical deactivation or steric hindrance at the active sites. Quality control testing of the manufactured hard gelatin capsules confirmed high dosing precision and a rapid *in vitro* disintegration time, driven by the synergistic wicking and swelling actions of the filler and superdisintegrant. Overall, these findings validate the technological and biopharmaceutical feasibility of the formulated capsules, offering a highly uniform and bioactive oral delivery system designed to counter reactive oxygen species.

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Availability of data and materials

The data generated in the present study are included in the figures and/or tables of this article. The other data generated in the present study may be requested from the corresponding author.

Ethics approval and consent to participate

Not applicable.

AI use disclosure

Not applicable.

Conflict of interest

The authors declare no conflict of interest.

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